

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for identifying a stabilized aptamer that binds to a target molecule, wherein the aptamer comprises at least one 2'-OH guanosine, at least one 2-OMe guanosine, and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH), comprising the steps:

- a) preparing a transcription reaction mixture comprising (i) a ~~modified double-mutant T7 RNA polymerase that comprises at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase~~ consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine, (ii) one or more 2'-OMe modified nucleotide triphosphates (2'-OMe NTPs), including at least one 2'-OMe GTP, (iii) 2'-OH guanosine triphosphate, (iv) magnesium ions, (v) manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, and (vi) one or more double-stranded oligonucleotide transcription templates, wherein the modified RNA polymerase exhibits an increased ability to incorporate 2'-OMe NTPs as compared to the ability of the corresponding unmodified RNA polymerase to incorporate 2'-OMe NTPs;
- b) preparing a candidate mixture of stabilized single-stranded nucleic acids by transcribing the one or more oligonucleotide transcription templates under conditions whereby the ~~double-mutant T7~~ modified RNA polymerase incorporates the 2'-OMe NTPs, including at least one 2'-OMe GTP, into the stabilized single-stranded nucleic acids of the candidate mixture, wherein the stabilized single-stranded nucleic acids have a length in the range of 30-50 nucleotides;
- c) contacting the candidate mixture with the target molecule;
- d) partitioning the stabilized single-stranded nucleic acids having an increased affinity to the target molecule relative to the nucleic acids from the remainder of

the candidate mixture; and

- e) amplifying the increased affinity stabilized single-stranded nucleic acids, in vitro, using the transcription reaction mixture of step a) to generate a ligand-enriched mixture of nucleic acids, whereby stabilized aptamers comprising at least one 2'-OMe GTP are identified.

2 - 100. (Cancelled).

101. (Currently Amended) A method for identifying a stabilized aptamer that binds to a target molecule, wherein the aptamer comprises at least one 2'-OH guanosine, at least one 2-OMe guanosine, and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH), comprising the steps:

- a) preparing a transcription reaction mixture comprising (i) a ~~modified double-mutant T7~~ RNA polymerase that ~~comprises at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase~~ consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine, (ii) one or more 2'-OMe modified nucleotide triphosphates (2'-OMe NTPs), including at least one 2'-OMe GTP, (iii) 2'-OH guanosine triphosphate, (iv) magnesium ions, (v) manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, and (vi) one or more oligonucleotide transcription templates, wherein the modified RNA polymerase exhibits an increased ability to incorporate 2'-OMe NTPs as compared to the ability of the corresponding unmodified RNA polymerase to incorporate 2'-OMe NTPs;
- b) preparing a candidate mixture of stabilized single-stranded nucleic acids by transcribing the one or more oligonucleotide transcription templates under conditions whereby the ~~double-mutant T7~~ modified RNA polymerase incorporates the 2'-OMe NTPs, including at least one 2'-OMe GTP, into the stabilized single-stranded nucleic acids of the candidate mixture, wherein the stabilized single-

- stranded nucleic acids have a length in the range of 30-50 nucleotides;
- c) contacting the candidate mixture with the target molecule;
 - d) partitioning the stabilized single-stranded nucleic acids having an increased affinity to the target molecule relative to the nucleic acids from the remainder of the candidate mixture; and
 - e) amplifying the increased affinity stabilized single-stranded nucleic acids, in vitro, using the transcription reaction mixture of step a) to generate a ligand-enriched mixture of nucleic acids, whereby stabilized aptamers comprising at least one 2'-OMe GTP are identified.

102. (Currently Amended) A method for transcribing a stabilized oligonucleotide wherein the oligonucleotide comprises at least one 2'-OH guanosine, at least one 2-OMe guanosine, and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (π /mGmH), comprising the steps:

- a) preparing a transcription reaction mixture comprising (i) a ~~modified double-mutant T7 RNA polymerase that comprises at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase~~ consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine, (ii) one or more 2'-OMe modified nucleotide triphosphates (2'-OMe NTPs), including at least one 2'-OMe GTP, (iii) 2'-OH guanosine triphosphate, (iv) magnesium ions, (v) manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, and (vi) one or more double-stranded oligonucleotide transcription templates, wherein the modified RNA polymerase exhibits an increased ability to incorporate 2'-OMe NTPs as compared to the ability of the corresponding unmodified RNA polymerase to incorporate 2'-OMe NTPs; and
- b) transcribing the one or more oligonucleotide transcription templates under conditions to generate a stabilized transcribed oligonucleotide, whereby the

~~double-mutant T7~~modified RNA polymerase incorporates the 2'-OMe NTPs, including at least one 2'-OMe GTP, into the stabilized transcribed oligonucleotide, and wherein the stabilized transcribed oligonucleotide has a length in the range of 30-50 nucleotides.

103. - 181. (Cancelled).

182. (Currently Amended) A method for transcribing a stabilized oligonucleotide wherein the oligonucleotide comprises at least one 2'-OH guanosine, at least one 2-OMe guanosine, and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH), comprising the steps:

- a) preparing a transcription reaction mixture comprising (i) a ~~modified double-mutant T7~~ RNA polymerase that ~~comprises at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase~~ consists of a Y639F/H784A mutant T7 RNA polymerase in which the tyrosine residue at position 639 has been changed to a phenylalanine and the histidine residue at position 784 has been changed to an alanine, (ii) one or more 2'-OMe modified nucleotide triphosphates (2'-OMe NTPs), including at least one 2'-OMe GTP, (iii) 2'-OH guanosine triphosphate, (iv) magnesium ions, (v) manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, and (vi) one or more oligonucleotide transcription templates, wherein the modified RNA polymerase exhibits an increased ability to incorporate 2'-OMe NTPs as compared to the ability of the corresponding unmodified RNA polymerase to incorporate 2'-OMe NTPs; and
- b) transcribing the one or more oligonucleotide transcription templates under conditions to generate a stabilized transcribed oligonucleotide, whereby the ~~double-mutant T7~~modified RNA polymerase incorporates the 2'-OMe NTPs, including at least one 2'-OMe GTP, into the stabilized transcribed oligonucleotide, and wherein the stabilized transcribed oligonucleotide has a length in the range of 30-50 nucleotides.

183. - 188. (Cancelled).

189. (Previously Presented) The method of claim 1, wherein the method further comprises the step:

f) repeating steps c), d) and e) wherein the candidate mixture of step c) is the ligand-enriched mixture of nucleic acids from step e).

190. (Previously Presented) The method of claim 101, wherein the method further comprises the step:

f) repeating steps c), d) and e) wherein the candidate mixture of step c) is the ligand-enriched mixture of nucleic acids from step e).

191. - 194. (Cancelled).

195. (Previously Presented) The method of claim 1 or claim 101, wherein at least 80% of the guanosine triphosphate nucleotides in the stabilized aptamers are 2'-OMe GTP and the remaining guanosine triphosphate nucleotides in the stabilized aptamers are 2'-OH guanosine triphosphate nucleotides.

196. (Previously Presented) The method of claim 102 or claim 182, wherein at least 80% of the guanosine triphosphate nucleotides in the stabilized transcribed oligonucleotide are 2'-OMe GTP and the remaining guanosine triphosphate nucleotides in the stabilized transcribed oligonucleotide are 2'-OH guanosine triphosphate nucleotides.

197. (New) The method of claim 1, 101, 102 or 182, wherein the oligonucleotide transcription template further comprises a leader sequence incorporated into a fixed region at the 5' end of the template.

198. (New) The method of claim 101 or claim 182, wherein the oligonucleotide transcription template is double-stranded.

199. (New) The method of claim 197, wherein the leader sequence comprises an all-purine leader sequence.

200. (New) The method of claim 199, wherein the all-purine leader sequence has a length selected from the group consisting of: at least 8 nucleotides long, at least 10 nucleotides long, at least 12 nucleotides long and at least 14 nucleotides long.

201. (New) The method of claim 1, 101, 102 or 182, wherein each NTP, 2'-modified NTP or 2'-OMe NTP is present at a concentration of about 0.5 mM, the concentration of magnesium ions is about 5.0 mM and the concentration of manganese ions is about 1.5 mM.

202. (New) The method of claim 1, 101, 102 or 182, wherein each NTP, 2'-modified NTP or 2'-OMe NTP is present at a concentration of about 1.0 mM, the concentration of magnesium ions is about 6.5 mM and the concentration of manganese ions is about 2.0 mM.

203. (New) The method of claim 1, 101, 102 or 182, wherein each NTP, 2'-modified NTP or 2'-OMe NTP is present at a concentration of about 2.0 mM, the concentration of magnesium ions is about 9.6 mM and the concentration of manganese ions is about 2.9 mM.

204. (New) The method of claim 1, 101, 102 or 182, wherein the transcription reaction mixture further comprises GMP.

205. (New) The method of claim 1, 101, 102 or 182, wherein the transcription reaction mixture further comprises polyalkylene glycol.

206. (New) The method of claim 205, wherein the polyalkylene glycol is polyethylene glycol.

207. (New) The method of claim 1, 101, 102 or 182, wherein the 2'-OMe modified nucleotide triphosphates comprise a mixture of 2'-OMe adenosine triphosphate, 2'-OMe cytidine

triphosphate, 2'-OMe uridine triphosphate, 2'-OMe guanosine triphosphate and 2'-OH guanosine triphosphate, wherein the 2'-OH guanosine triphosphate comprises a maximum of about 10% of the total guanosine triphosphate population.

208. (New) The method of claim 1, 101, 102 or 182, wherein the transcription reaction mixture further comprises spermidine, spermine, or both spermidine and spermine.

209. (New) The method of claim 1, 101, 102 or 182, wherein the concentration of magnesium ions is between 3.0 and 3.5 times greater than the concentration of manganese ions.

210. (New) The method of claim 1, 101, 102 or 182, wherein the transcription reaction mixture further comprises inorganic pyrophosphatase.